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Effects of Rewarding and Unrewarding Experiences on the Response to Host-induced Plant Odors of the Generalist Parasitoid *Cotesia marginiventris* (Hymenoptera: Braconidae)

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Abstract Associative learning is known to modify foraging behavior in numerous parasitic wasps. This is in agreement with optimal foraging theory, which predicts that the wasps will adapt their responses to specific cues in accordance with the rewards they receive while perceiving these cues. Indeed, the generalist parasitoid *Cotesia marginiventris* shows increased attraction to a specific plant odor after perceiving this odor during contact with hosts. This positive associative learning is common among many parasitoids, but little is known about the effects of unrewarding host searching events on the attractiveness of odors. To study this, preferences of female *C. marginiventris* for herbivore-induced odors of three plant species were tested in a six-arm olfactometer after the wasps perceived one of these odors either i) without contacting any caterpillars, ii) while contacting the host caterpillar *Spodoptera littoralis*, or iii) while contacting the non-host caterpillar *Pieris rapae*. The results confirm the effects of positive associative learning, but showed no changes in innate responses to the host-induced odors after "negative" experiences. Hence, a positive association is made during an encounter with hosts, but unsuccessful host-foraging experiences do not necessarily lead to avoidance learning in this generalist parasitoid.

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Introduction

Learning allows foraging insects to evaluate the profitability of their environment and to optimize the exploitation of unpredictably distributed resources (Menzel et al. 1993). This is also expected to be the principal function of associative learning in insect parasitoids (Turlings et al. 1993; Vet et al. 1995; Steidle and van Loon 2003), but conclusive evidence is lacking. Parasitoids of herbivores have the arduous task of finding their usually inconspicuous hosts in complex vegetation (Godfray and Waage 1988). Herbivores will have undergone selection to limit chemical emissions in order to avoid being detected by their natural enemies (Tumlinson et al. 1992; Vet and Dicke 1992; Stowe et al. 1995). These enemies appear to have found a solution by using plant-provided signals in their search for preys or hosts (Dicke and Sabelis 1988; Turlings et al. 1990a, 1995). The signals come in the form of volatile organic compounds emitted by plants in response to herbivore damage, and play key roles in numerous tritrophic interactions (for reviews, see Vet and Dicke 1992; Dicke and Vet 1999; Turlings and Benrey 1998; Turlings and Wäckers 2004). Plants under herbivore attack emit the volatiles systemically and in large amounts, thus providing parasitoids with olfactory signals that can be readily detected over larger distances.

However, host-induced plant odors can be highly variable and parasitic wasps have to optimize their use of the available signals within the context of this variability. Genotype, plant stage and environmental conditions are all known to influence the emission of host-induced odors (Loughrin et al. 1995; Krips et al. 2001; Hoballah et al. 2002; Gouinguené and Turlings 2002; Degen et al. 2004). In addition, the makeup of the blend produced by a plant species may depend on the herbivore species (Powell et al. 1998; De Moraes et al. 1998), or even the stage of the attacking herbivore (Takabayashi et al. 1995). Whatever the source of variability, parasitoids are very flexible in their responses to long-range cues and this adaptability may help them to focus on those cues that are most reliably associated with host presence (Vet and Dicke 1992; Wäckers and Lewis 1994). Indeed, parasitic wasps can make an association between an encounter with a suitable host and the odor that they perceive during such an encounter. Subsequently, their response to the learned odor is increased (Turlings et al. 1993; Vet et al. 1995).

If indeed associative learning enhances the efficiency of parasitoids in their foraging efforts, then it can be expected that negative or unrewarding experiences also have an impact on their foraging decisions. A wasp might learn to avoid an odor that it perceives in an unprofitable microhabitat. This is not the case for the generalist ectoparasitoid *Exeristes roborator*, which shows no change its odor preference after repeated exposures to a particular odor without encountering hosts (Wardle and Borden 1989). In contrast, females of the generalist *Leptopilina heterotoma* can learn to distinguish between the odors of profitable and unprofitable host habitats (Papaj et al. 1994; Vet et al. 1998). Visual cues may also be interpreted negatively if they are associated with unsuitable hosts, as has been shown for the

tachinid fly *Exorista mella* (Stireman 2002). Takasu and Lewis (2003) found that females of the specialist parasitoid *Microplitis croceipes* that have oviposited in a lepidopteran species that is not a host cease to respond to a previously learned odor; they also decrease subsequent responses to general odors. Similarly, Lizuka and Takasu (1998) found that females of a pupal parasitoid, *Pimpla luctuosa*, cease to respond to a learned odor after having probed "simulated" hosts with their ovipositor.

As is expected for positive learning (Vet and Dicke 1992; Steidle and van Loon 2003), the ability to use negative information in foraging efforts may differ between generalist and specialist parasitoids. We predict that encounters with non-host insects in association with a specific plant odor will have a particularly negative effect if there is a high likelihood that subsequent responses to the experienced odor will again lead them to non-hosts. As yet, this has not been tested for generalist wasps that use host-induced volatiles from various plant species to locate hosts.

Here we present three experiments with the generalist parasitoid *Cotesia marginiventris* that studied the effects of unrewarding experiences and experiences with non-hosts on the parasitoid's subsequent responsiveness to host-induced plant odors perceived during these experiences. It is known that females of *C. marginiventris* readily associate rewarding experiences (i.e.: contact with host feces or ovipositions in a suitable host) with a plant odor, and they show an increased attraction to a rewarding odor (Turlings et al. 1989, 1990a, b; Hoballah and Turlings 2005; Tamò et al. 2006). Using a six-arm olfactometer we tested how the wasp's response to the induced odor of three plant species (maize, cotton and cowpea) is affected by positive (contact with the host *Spodoptera littoralis*) and negative (no host contact or contact with the non-host *Pieris rapae*) experiences.

We aim to answer two questions with these experiments: (1) Does an exposure to an host-induced plant odor in the absence of hosts affect the subsequent attraction of *C. marginiventris* to this odor? (2) What is the effect of contact with a non-host species in the presence of a host-induced plant odor on a generalist wasp's odor preferences? Results confirm that positive experiences increase responsiveness to the experienced odor, whereas negative experiences in these experiments altered neither the responsiveness nor the odor preferences of *C. marginiventris*.

Materials and Methods

Plants

We compared the attractiveness of host-induced plant volatiles (HIPVs) produced by three different plant species: maize (*Zea Mays*, var. Delprim), cotton (*Gossypium hirsutum*, var. "Coton en pots") and cowpea (*Vigna unguiculata*, var. "black-eyes", Haefliger AG/SA). Plants were grown from seed in plastic pots (8 cm diameter, 6 cm high) filled with fertilized commercial soil (Coop, Switzerland) in a climate chamber (23°C, 60% R.H., Photoperiod: 16L/8D, 50,000 lm/m^2) and were watered every day. When maize seedlings were 10–12 days old and when cotton and cowpea seedlings were 15–17 days old (all the plants had three fully developed leaves), they were taken out of the climate chamber in the evening, placed under ambient laboratory conditions and then prepared as follows to serve the next day as odor sources. Two

seedlings from each plant species were carefully transplanted together into a glass pot (5 cm diameter, 11 cm high) and the pot was covered with a glass vessel of the olfactometer (Fig. 1). Two seedlings of the same plant species were infested with twenty 2nd instar *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) larvae deposited from the top opening of the vessel. Each such vessel could either be used as an odor source to test in the olfactometer or to expose the wasps to the host-induced plant odor before the test.

Insects

Parasitoids

The endoparasitoid *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae) is a generalist that has a wide range of hosts. This includes at least twenty species from the Noctuidae, including *Spodoptera spp* (Krombein et al. 1979; Jalali et al. 1987). Yet, this parasitoid cannot successfully develop on *Pieris rapae* larvae (Personal observation). We reared *C. marginiventris* wasps from a culture that originated from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA), and that was frequently refreshed with wild individuals to preclude inbreeding effects. Female wasps were reared in the laboratory on *Spodoptera littoralis* (Hoballah et al. 2002). Adults were kept in plastic cages (30×30×30 cm) stored in an incubator (25°C and 16L/8D) and provided with honey and moist cotton wool. We used 2–4 day-old mated females in all experiments.

Hosts

Eggs of *S. littoralis* supplied by Syngenta (Stein, Switzerland) were incubated each week on moist filter paper. Emerged caterpillars were fed with a wheatgerm-based

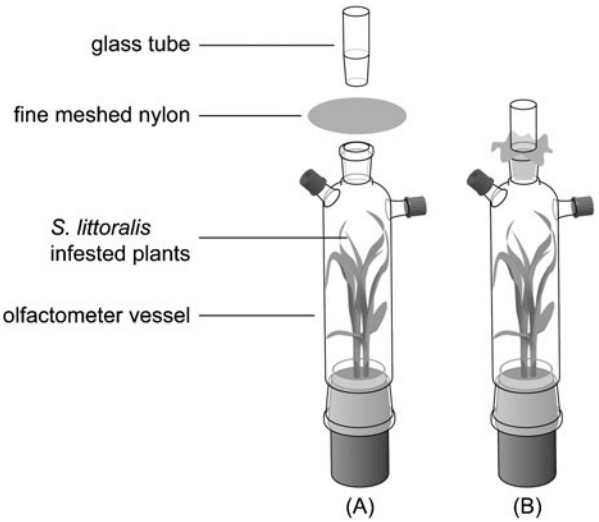

Fig. 1 System used to give female *C. marginiventris* prior experience (**A**) Vessel containing two *Spodoptera* infested maize plants (**B**) Full system operational (Drawing : T. Degen).

artificial diet provided by Syngenta and kept in plastic-boxes (15×9×5 cm) under ambient laboratory conditions. Second instar larvae were used in all the experiments, either to infest plants or to give female wasps an oviposition experience.

Non-hosts

Larvae of the non-host *Pieris rapae* L. (Lepidoptera: Pieridae) were obtained from a colony at the University of Lausanne that was reared on cabbage. For the experiments, they were transferred from cabbage to the experience tube above their assigned experimental plant (Fig. 1). To determine if the wasps recognize these larvae as unsuitable, we conducted preliminary tests by exposing female *C. marginiventris* to second instar *P. rapae* larvae (non-host) and to second instar *S. littoralis* larvae (host), while they perceived the HIPVs of maize (see Fig. 1 and "Experiencing the wasps"). The larvae were frequently probed by *C. marginiventris*, but not as readily as larvae of the host *S. littoralis*. Dissections of *S. littoralis* and *P. rapae* second instar larvae were made just after contact they had been contacted by a wasp. Only about 30% of the probings in the non-host led the wasp to lay an egg, whereas a great majority of females (92%) had oviposited in *S. littoralis*. Similar tests showed that also when female *C. marginiventris* were not exposed to any HIPVs they would in most cases not lay an egg in *P. rapae*. Thus, the outcomes of an attack in *S. littoralis* and *P. rapae* were clearly different and the wasp must have been able to distinguish between them.

Giving the Wasps Experience

Before their release in the olfactometer, female *C. marginiventris* were twice given an experience, once the day before around 16h00 and once one hour before the test. For this "training" experience, we used the same type of vessel with host-induced plants (Fig. 1) as used for the olfactometer tests (see: "Plants"). The top openings of these vessels were connected to a 2.5 cm diam. glass tube that contained a fine meshed nylon that prevented wasps and larvae from entering the vessel. Wasps were individually placed in the tube (with or without larvae) and the top of the tube was then covered by a transparent lid. Wasp behavior was observed through this lid. Female wasps were trained in groups of two or three in order to have at least six individuals of each experience type prepared for the olfactometer tests. In the tube, the wasps were exposed to the *Spodoptera*-induced odor of one of the three plant species and were offered either (i) no larvae, (ii) host larvae (*S. littoralis*), or (iii) non-host larvae (*P. rapae*). As an additional control, a group of females were kept as naives (without host or plant odor experience).

Behavioral Tests: The Six-arm Olfactometer

Odor preferences were tested in the six-arm olfactometer described in Turlings et al. (2004). The setup consists of a construction with three shelves: the top shelf carries the olfactometer, the middle shelf is used to release the insects tested and the bottom shelf holds the odor sources. On the bottom shelf pure humidified air is pushed into the lower part of each odor source vessel (1.2 l/min). Half of the airflow (0.6 l/min)

is pulled out through a filter attached to an opening at the upper part of the vessel. This filter can be extracted after an experiment for subsequent chemical analyses (Turlings et al. 2004). The remaining air (0.6 l/min) from each vessel is carried via a Teflon tube to an arm of the olfactometer. This way, all six airflows come together in a central glass chamber, in which the wasps are released and can make their choice for an odor by walking into one of the arms. After walking into an arm they end up in a trapping bulb, where they can be readily counted (see Turlings et al. (2004) for further details). In our tests, the wasps always had a choice between three arms connected to host-induced plants odors of maize, cotton, and cowpea alternated with three arms connected to empty vessels. The positions of maize, cotton, and cowpea were randomly assigned for each different experimental day.

Female wasps were released in groups of six inside the central chamber, one hour after having received their second experience. The sequences in which the different experience groups were released were randomized to avoid possible daytime effects. We let the females choose during a period of 30 min, after which the six individuals were removed from the device with an aspirator. The number of females having made a choice for each plant was recorded, thus establishing the relative preference of a treatment group. The first experiment was replicated on seven days and the two other experiments on eight days. All experience treatments were tested once on each day with the same odor sources. At the end of a day, all parts of the olfactometer were washed with water and rinsed with acetone and hexane. After the solvents had evaporated in a fume hood the glass parts were dried in an oven at 250°C.

Experiment 1: The Effect of Failure to Find Hosts

In the first experiment, we assessed the effect of unrewarding experiences (defined as a failure to find a host in the presence of a particular odor). We compared the responses of females having performed two ovipositions in *S. littoralis* larvae (referred to as +) with those having encountered no larvae in the experience tube during 5 min (referred to as 0). We used maize (M) and cotton (Ct) for both (+) and (0) treatments. Including the group of naives (N), we tested five groups of wasps (M+/M0/Ct+/Ct0/N).

Experiment 2: The Effect of Encounters with Non-hosts

Given the results of the first experiment, we then evaluated the impact of an encounter with non-hosts on the wasps' responses to host-induced odors. Since a large majority (70%) of females did not lay eggs inside the non-host, the encounters were likely to be perceived as negative in terms of host search, which might lead to an avoidance of the odor detected during the encounter. Females having had such a negative experience are referred to as (−). Females (−) were either exposed to host-induced odors of maize (M−) or cotton (Ct−). We compared the olfactometer responses of wasps experiencing these unfruitful attacks with wasps that underwent the five treatments that were used in the first test. Thus, the preferences of seven groups of wasps were assessed in this second experiment (M+/M0/M−/Ct+/Ct0/Ct−/N).

Experiment 3: Including Experiences with Cowpea Odors

In this last experiment, we performed the same tests as in the previous experiment, except that we used the host-induced odors of cowpea (Cp) instead of cotton for the training. The reason for this is that infested cowpea seedlings were found to be the most attractive to naive wasps and avoidance after negative experience might be more apparent for the initially attractive cowpea volatiles. Hence, for this third experiment we compared the responses of seven groups of wasps, referred to as M+/M0/M−/Cp+/Cp0/Cp−/N.

Statistics

To compare the percentage of wasps responding to plant odors in different groups of females (having a different training), we performed a so-called Monte Carlo exact test. Let y_{ij} denote the counts in a two-way contingency table, with i labeling the rows and j the columns. Independence of the row and column classifications is very often tested using the usual Pearson statistic, often called the chi-squared statistic, which may be written $X^2 = \sum_{ij}\left(y_{ij} - y_{i\cdot}y_{\cdot j}/n\right)^2/\left(y_{i\cdot}y_{\cdot j}/n\right)$, where $y_{i\cdot}$ and $y_{\cdot j}$ are respectively the totals for the i^{th} row and j^{th} column, and n is the total for the entire table. Conventionally the hypothesis of row-column independence is rejected when X^2 is large compared to a chi-squared distribution, but if the cell counts are very small this provides a poor test, because the chi-squared distribution is then inappropriate. In our experiments, there were just six individuals for each sequence of releases, and our data often showed a probability of 1 for a wasp to respond, so use of a chi-squared distribution would be very misleading. Under a Monte Carlo exact test the conditional distribution of the test statistic, which provides an exact significance level, is approximated using a Metropolis–Hastings algorithm. We applied this approach using the deviance or likelihood ratio statistic $W = 2\sum_{i,j} y_{ij}\log\left\{n\,y_{ij}/y_{i\cdot}y_{\cdot j}\right)\}^2/\left(y_{i\cdot}y_{\cdot j}/n\right)$ rather than X^2 because W should have slightly higher power. Monte Carlo simulation of this sort is widely used to approximate exact tests in small samples or other situations where the standard chi-squared approximation for a test of fit is invalid; for more details see Davison and Hinkley (1997, Ch. 4.2.2). In this our case, the null hypothesis H_0 was that the number of wasps making a choice is independent of prior experience. The observed value W_{obs} of W was compared to 15,000 values simulated under H_0. We rejected H_0 at significance level $t\times100\%$ if the quantile Q_{1-t} of the simulated values was strictly inferior to W_{obs}, i.e. if $Q_{1-t} < W_{\text{obs}}$. We fixed the significance level at 5%, so all calculated quantiles were $Q_{0.95}$.

In the second part of the analysis, we created a log-linear model slightly modified from that of Tamò et al. (2006) to compare the relative attractiveness of a particular plant odor depending upon the wasps' prior experience. We also took into account the significant overdispersion of the data previously observed (Davison 2003; Turlings et al. 2004; Tamò et al. 2006) by using a stochastic version of the model. Our model also considered the censored nature of our data since not all the wasps made a choice during the 30 min (Ricard and Davison 2007).

We developed several models for different scenarios (Table 1) and compared them using likelihood ratio methods. All these models relied on the assumption

Table 1 Summary of the Four Log-linear Models Developed to Compare the Relative Attractiveness of a Particular Plant Odor Depending Upon Female *C. marginiventris* Prior Experience

Model	Tested hypothesis	Biological significance
1	(+) = N = (0)	No effect of wasp prior experience
2	(+) > (N) = (0)	Effect of positive associative learning (contact with a host increases wasp response)
3	(+) > N > (0)	Failure to find a host reduces female wasp response to the plant odor while contact with a host increases wasp response
4	(+) > N > (−)	Contact with a non-host reduces female wasp response to the plant odor while contact with a host increases wasp response

N = naive females, (+) = female wasps that had oviposited twice in a host (*S. littoralis*), (0) female wasps that found no host, and (−) = wasps that attacked a non-host (*P. rapae*)

that responses toward empty (*e*) arms could be different from those connected to a plant odor (p).

Our first model (1), which assumed that the attractiveness toward an arm was not modified by the prior wasp experience, was described by the equation

$$\log \lambda_{p+} = \log \lambda_p = \beta_p \ (\text{with p} = \{\text{maize, cotton, cowpea, empty arm}\}), \qquad (1)$$

where parameter β_p represents the measurement of the relative attractiveness of the odor source λ_p in the arm containing the plant *p*, while λ_{p+} corresponds to the relative attractiveness of a plant odor experienced by a wasp (+).

In model (2), the assumption was that wasps having treatments (+) were more attracted by the plant they have experienced positively. The increased attractiveness is described by parameter γ_p, with $\gamma_p > 0$:

$$\begin{aligned} \log \lambda_{p+} &= \beta_{p+} = \beta_p + \gamma_p, \\ \log \lambda_p &= \beta_p. \end{aligned} \qquad (2)$$

In model (3), we assumed that wasps (+) were more attracted to the plant they had already experienced compared to naive wasps that were more attracted to the plant than wasps (0):

$$\begin{aligned} \log \lambda_{p+} &= \beta_{p+} = \beta_p + \sigma_p + \gamma_p, \\ \log \lambda_{pN} &= \beta_{pN} = \beta_p + \sigma_p, \\ \log \lambda_{p0} &= \beta_p. \end{aligned} \qquad (3)$$

The difference of attractiveness for wasps (0) and naive wasps (N) was represented by parameter σ.

Finally, in model (4), the assumption was that wasps (−) were less attracted to the plant they have experienced negatively. The two first equations of model (3) remained unchanged, but the last equation was modified to

$$\log \lambda_{p-} = \beta_p. \qquad (4)$$

A significant difference between the fits of models (1) and (2) would mean that wasps (+) are more attracted to the plant odors they have experienced. If models (2) and (3) are different, this would show that wasps (0) respond significantly less to the

odors they have been exposed to. A difference between models (2) and (4) would indicate that wasps (−) are less attracted to the plant odors they have experienced. For each model, effects of experience were tested first as a global effect on both plants and then on each plant separately. All programs used in the present analysis were written in the software R (http://stat.ethz.ch/CRAN/).

Results

Experiment 1

Most of the wasps (77.2%) responded to HIPVs during this first experiment, thus confirming the suitability of the set-up for assessing the wasp' responses. The Metropolis–Hastings algorithm yielded a quantile of simulated deviances ($Q_{0.95}$= 49.2) lower than the observed deviance of the data (W_{obs}=55.8), thus indicating that there was a significant difference in responsiveness (proportion of wasps entering an arm) between the different wasp treatments. The most responsive wasps were those having a positive experience on cotton (C+), while the least responsive were those that failed in finding a host on cotton (C0) or had a positive experience on maize (M+) (Fig. 2).

Females that had a positive experience in the presence of induced plant odor displayed a highly significant increase in response towards the odor ($Chi_1^2 = 17.39$; $P<0.0001$). The effect of positive experience on Ct+ females was highly significant ($Chi_1^2 = 18.56$; $P<0.0001$). Although their response was highest (Fig. 3), M+ females were not significantly more attracted to maize than naïve (N) ones ($Chi_1^2 = 3.22$; $P=0.07$). There was also no difference in responses between naive females and females that perceived the HIPVs in the absence of hosts (M0: $Chi_1^2 = 2.02$; $P=0.15$ and Ct0: $Chi_1^2 = 0.99$; $P=0.32$).

Model (2) provided the best fit to the data among the models fitted, thus showing that increased attraction occurring after a positive experience on a particular plant odor. Wasps were overall more attracted by cowpea than by the other two plant ($P<0.0001$).

Experiment 2

In *Experiment 2*, we compared the same treatments as in *Experiment 1*, but we also included groups of females that contacted the non-host *P. rapae* in presence of maize (M−) or cotton (Ct−) volatiles. A majority of females (80.7% on average) chose an arm with a plant odor, and there was no significant difference in overall responsiveness between different groups of females (W_{obs}=66.1<$Q_{0.95}$=82.7). Among the three host-induced plant odors offered, cowpea odor was again significantly preferred ($P<0.0001$).

M+ females ($Chi_1^2 = 15.41$; $P<0.0001$) and Ct+ females ($Chi_1^2 = 25.5$; $P<0.0001$) exhibited a highly significant preference for the plant odor they had previously experienced (Fig. 4). Females having encountered *P. rapae* did not change their preference whether they experienced the odor of maize ($Chi_1^2 = 0.76$; $P=0.38$) or cotton ($Chi_1^2 = 1.83$; $P=0.18$). As in *Experiment 1*, females that did not

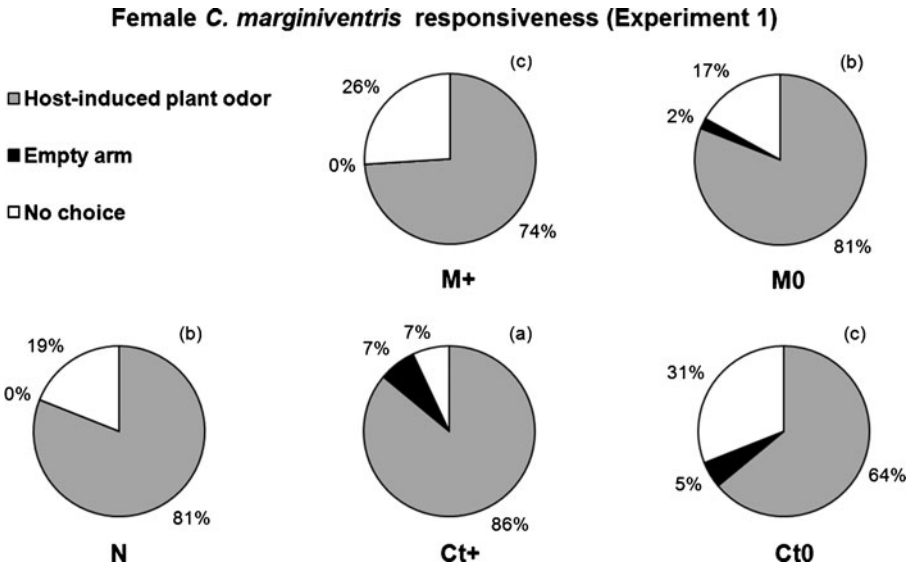

Fig. 2 Responsiveness of female *C. marginiventris* during *Experiment 1*. Five groups of wasps were tested: naive wasps (N), wasps that had oviposited in a host while exposed to maize (M+) or cotton (Ct+) volatiles, wasps that found no host while being exposed to maize (M0) or cotton (Ct0) volatiles. *Numbers* indicate the percentages of wasps entering an arm with host-induced plant odor entering an empty arm, or making no choice. *Letters* indicate groups with significant differences based on an exact test between simulated and observed deviances (Metropolis-Hasting algorithm).

encounter hosts while perceiving an odor also did not change their preference, whether the perceived odor was maize ($Chi_1^2 = 2.21$; P=0.14) or cotton ($Chi_1^2 = 0.06$; P=0.81).

Since models (3) and (4) did not provide a better fit, we found that model (2) again best described the wasp responses, confirming the strong and highly significant increase of odor attractiveness observed after encountering a host.

Experiment 3

A very high proportion (84.2% in average) of wasps responded by entering one of the arms with a plant odor, and no significant difference of overall responsiveness

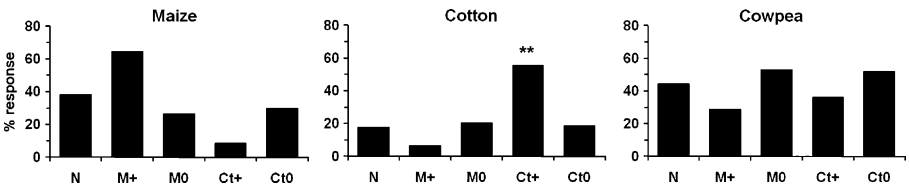

Fig. 3 Female *C. marginiventris* olfactory preference to host-induced plant volatiles from maize, cotton, and cowpea in *Experiment 1*. Five groups of wasps were tested: naive wasps (N), wasps that had oviposited in a host while exposed to maize (M+) or cotton (Ct+) volatiles, wasps that found no host while being exposed to maize (M0) or cotton (Ct0) volatiles. *Asterisk* indicates significant differences (** P< 0.01; * P<0.05) between the response of an experienced group and naive wasps. Data were analyzed with likelihood ratio tests performed on log linear models (see text for more details).

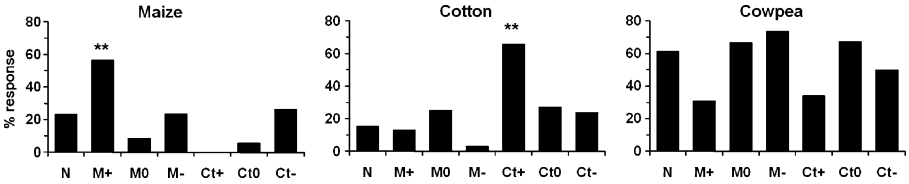

Fig. 4 Female *C. marginiventris* olfactory preference to host-induced plant volatiles from maize, cotton, and cowpea in *Experiment 2*. Seven groups of wasps were tested: naive wasps (N), wasps that had oviposited in a host while exposed to maize (M+) or cotton (Ct+) volatiles, wasps that found no hosts while being exposed to maize (M0) or cotton (Ct0) volatiles, and wasps that had contacted a non-host while exposed to maize (M−) or cotton (Ct−) volatiles. *Asterisk* indicates significant differences (** $P<0.01$; * $P<0.05$) between the response of an experienced group and naive wasps. Data were analyzed with likelihood ratio tests performed on log linear models (see text for more details).

(Fig. 5) was found between the treatment groups ($W_{obs}=71.5<Q_{0.95}=76.2$). A great majority of naive wasps (74.4%) entered the arm with cowpea volatiles during this last experiment and this preference was highly significant ($P<0.0001$).

Experienced wasps were exposed to either maize or cowpea odors. The analysis showed that in general, the experience significantly increased the attractiveness of an odor ($Chi_1^2 = 6.91$; $P<0.01$) (Fig. 6). M+ females significantly shifted their response towards maize ($Chi_1^2 = 5.27$; $P=0.02$). Cp0 and Cp− females respond slightly less to cowpea volatiles than naive ones (respectively 57% and 60%), but this difference was not significant ($Chi_1^2 = 1.07$; $P=0.30$ and $Chi_1^2 = 0.08$; $P=0.77$). Neither M0 females ($Chi_1^2 = 0.17$; $P=0.69$) nor M− females ($Chi_1^2 = 0.17$; $P=0.68$) reduced their response toward maize odor.

Model (2) provided finally the best fit relative to all other models, just as was observed in *Experiments 1* and *2*.

Discussion

The fitness of parasitoids is largely determined by the number of hosts a female can successfully parasitize during her lifetime. Hence, parasitic wasps are expected to have evolved foraging strategies that would optimize their chances to localize suitable hosts with minimal expenditure of time and energy. Associative learning, whereby female wasps increase their responsiveness to odor and visual cues that they perceive when they encounter hosts, is thought to be one such an evolved strategy (Turlings et al. 1993; Wäckers and Lewis 1994; Vet et al. 1995, 1998). For the same reason, we hypothesized that the failure to find hosts and unrewarding encounters with non-hosts should have the opposite effect, attenuating responses to associated odors. However, our results suggest that female *C. marginiventris* do not change their response towards HIPVs after unrewarding experiences; in none of the experiments was there a significant effect of an unrewarding experience on the responsiveness of the wasps as compared to that of naive wasps.

We purposely included an exceptionally attractive cowpea variety (D'Alessandro and Turlings 2005) in the olfactometer tests, assuming that a reduction in attractiveness would be most evident in case of a strong innate attractiveness. The high attractiveness was confirmed, with almost 75% of naive females choosing for

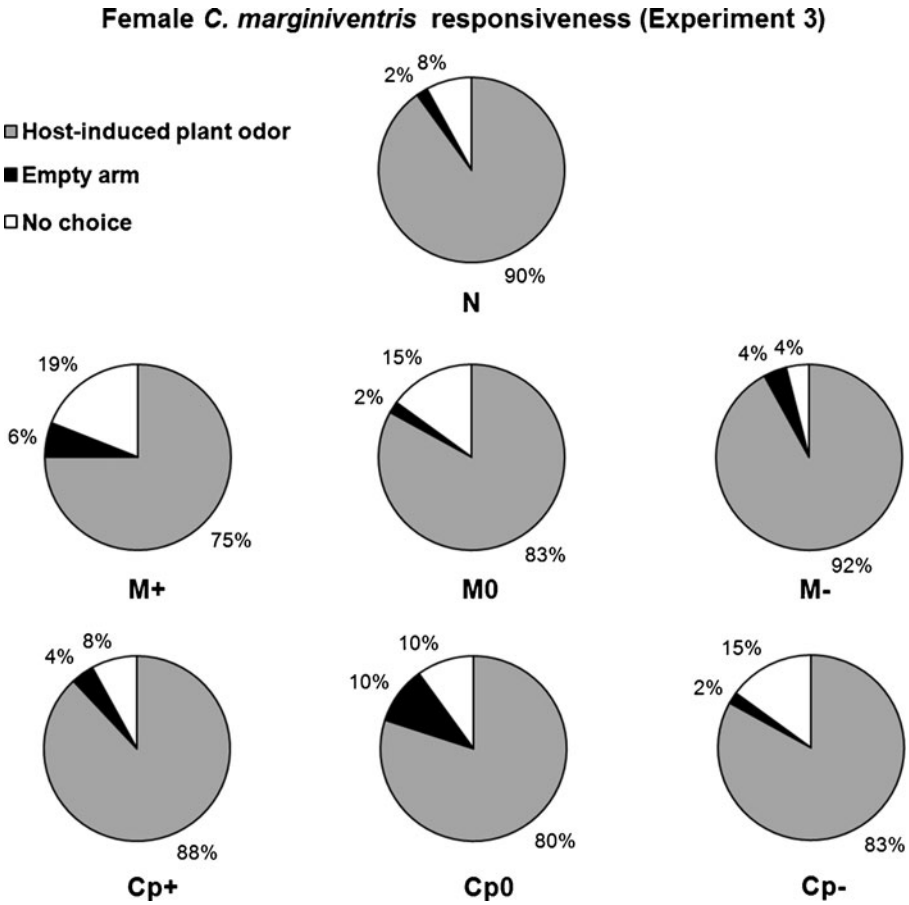

Fig. 5 Responsiveness of female *C. marginiventris* during *Experiment 3*. *Numbers* indicate the percentages of wasps entering an arm with a host-induced plant odor, entering an empty arm, or making no choice. No significant difference of responsiveness was found between groups of wasps.

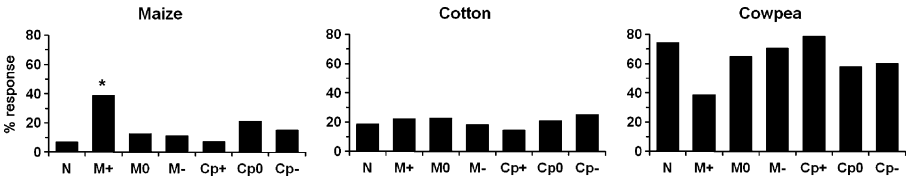

Fig. 6 Female *C. marginiventris* olfactory preference to host-induced plant volatiles from maize, cotton, and cowpea in *Experiment 3*. Seven groups of wasps were tested: naive wasps (N), wasps that had oviposited in a host while exposed to maize (M+) or cowpea (Cp+) volatiles, wasps that found no host while being exposed to maize (M0) or cowpea (Cp0) volatiles, and wasps that had contacted a non host while being exposed to maize (M−) or cowpea (Cp−) volatiles. *Asterisk* indicates significant difference (** $P<0.01$; * $P<0.05$) between the response of an experienced group and naive wasps. Data were analyzed with likelihood ratio tests performed on log linear models (see text for more details).

the cowpea odor. However, and despite a slight trend, neither of the two negative experiences significantly reduced this response to cowpea volatiles (Fig. 6). The strong attraction to the induced cowpea odor remains intriguing, as herbivore-damaged cowpea releases far less volatiles than cotton and maize (Hoballah et al. 2002; D'Alessandro and Turlings 2005; Tamò et al. 2006). This observation confirms the notion that minor compounds may be of key importance for the innate attraction of *C. marginiventris* (D'Alessandro and Turlings 2005).

The lack of an effect of unrewarding experiences suggests that *C. marginiventris* follows a similar strategy as observed for the generalist *Exeristes roborator*. This ectoparasitoid shows no decrease in responsiveness after several exposures to highly attractive apple scent without encountering hosts (Wardle and Borden 1989). However, the behavior of *C. marginiventris* seems to contrast with that of other parasitoids. For instance, female *Leptopilina heterotoma* avoid microhabitats associated with host absence (Papaj et al. 1994; Vet et al. 1998), whereas female *Microplitis croceipes* and *Pimpla luctuosa* avoid artificial odors like vanilla or strawberry after experiencing these odors during unsuccessful oviposition attempts (Takasu and Lewis 2003; Lizuka and Takasu 1998). The contrasting results of the above-mentioned studies might imply that different parasitoids employ different host foraging strategies. Indeed, some parasitic wasps such as *Campoletis sonorensis* do not learn HIPVs during rewarding experiences (McAuslane et al. 1991; Tamò et al. 2006), whereas other species such as *M. croceipes* readily learn to respond to any odor that is associated with hosts or food (Lewis and Takasu 1990).

Whereas non-host encounters or the absence of hosts in the presence of HIPVs did not alter the females' innate odor preferences, rewarding experiences with hosts significantly increased the preference for the experienced odor, as was demonstrated previously (Turlings et al. 1991; Tamò et al. 2006). Females that oviposited in *S. littoralis* larvae while perceiving the odor of host-damaged plants drastically increased their preference for the experienced odor, except for cowpea volatiles. The most spectacular shift of preference took place during the second experiment when the percentage of wasps attracted to cotton volatiles reached 66% for wasps having contacted hosts in the presence of host-damaged cotton odor (Ct+), while of the naive females (N) only 15% were attracted to this odor (Fig. 4). This effect of positive associative learning was obvious in each of the three experiments, and in most cases, the observed shift of preference was significant. This demonstrated effect of positive associative learning indicates that *C. marginiventris* females perceived the HIPVs emitted by the plant during the experience procedure. Hence, the absence of an effect of negative experiences cannot be explained by an inadequate training set up and the results also rules out the possibility that female wasps were not able to distinguish between the different HIPVs in the olfactometer.

One explanation for the contrasting results obtained for different parasitoids could be the different ways in which the wasps were trained. Indeed, *C. marginiventris* were here only confronted with rewarding *or* unrewarding situations before the olfactometer tests, whereas the effects of negative experiences on *L. heterotoma*, *M. croceipes* and *P. luctuosa* were mostly assessed in a previously rewarding microhabitat. The completeness of information is considered of primary importance for female parasitic wasps in order to evaluate the profitability of its environment (Vet et al. 1998). This notion is also in accordance with the above-mentioned results

for *E. roborator*, which also only received the negative experience (Wardle and Borden 1989). It is safe to conclude that *C. marginiventris'* innate response is not affected by an unrewarding response, but it remains possible that increased preference for an odor after a positive experience can be neutralized by an unrewarding experience with the same odor. This is exactly what experiments conducted with *M. croceipes* indicate; oviposition by this wasp in a non-host larva reduces the preference newly induced—either by associative learning or by sensitization—of vanilla odor (Takasu and Lewis 2003).

Finally, a recent study on how plant hoppers may affect the odor emission of *Spodoptera*-damaged maize plants (Erb et al. submitted) points at another possible reason why we did not find the hypothesized negative effect. It may be that an effect of negative association on the foraging behavior of *C. marginiventris* only takes place when the wasps contact non-hosts that they normally encounter in their natural environment. *Pieris rapae* will normally not be encountered by *C. marginiventris*, as *Pieris* species exclusively feed on Brassicacae plants, which do not support hosts of this generalist. Therefore, the parasitoid may not have evolved to recognize this non-host. In a follow-up study, we will explore the possibility that encounters of non-hosts that occur on plants frequently visited by *C. marginiventris* differentially affect the wasp's responsiveness.

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References

D'Alessandro M, Turlings TCJ (2005) In situ modification of herbivore-induced plant odours: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. Chem Senses 30:1–15

Davison AC (2003) Statistical models. Cambridge University Press, Cambridge

Davison AC, Hinkley DV (1997) Bootstrap methods and their application. Cambridge University Press, Cambridge

De Moraes CM, Lewis WJ, Paré PW, Alborn HT, Tumlinson JH (1998) Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573

Degen T, Dillmann C, Marion-Poll F, Turlings TCJ (2004) High genetic variability of herbivore-induced volatile emission within a broad range of maize inbred lines. Plant Physiol 135:1928–1938

Dicke M, Sabelis MW (1988) How plants obtain predatory mites as bodyguards. Neth J Zool 38:148–165

Dicke M, Vet LEM (1999) Plant-carnivore interactions: evolutionary and ecological consequences for plant, herbivore and carnivore. In: Olf H, Brown BK, Drent RH (eds) Herbivores: between plants and predators. Blackwell, Oxford, pp 483–520

Erb M, Foresti N, Turlings TCJ. A robust partnership: a tritrophic interaction resists disturbance by a phloem feeder. (submitted)

Godfray HCJ, Waage JK (1988) Learning in parasitic wasps. Nature 331:211

Gouinguené SP, Turlings TCJ (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol 129:1296–1307

Hoballah ME, Turlings TCJ (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. J Chem Ecol 31:2003–2018

Hoballah MEF, Tamò C, Turlings TCJ (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: is quality or quantity important? J Chem Ecol 28:951–968

Jalali SK, Singh SP, Ballal CR (1987) Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). Entomon 12:59–62

Krips OE, Willems PEL, Gols R, Posthumus MA, Gort G, Dicke M (2001) Comparison of cultivars of ornamental crop *Gerbera jamesonii* on production of spider mite-induced volatiles, and their attractiveness to the predator *Phytoseiulus persimilis*. J Chem Ecol 27:1355–1372

Krombein KV, Hurd PD, Smith JDR, Burks BD (1979) Catalog of Hymenoptera of America north of Mexico. Smithsonian Instit. Press, Washington

Lewis WJ, Takasu K (1990) Use of learned odours by a parasitic wasp in accordance with host and food needs. Nature 348:635–636

Lizuka T, Takasu K (1998) Olfactory associative learning of the pupal parasitoid *Pimpla luctuosa* Smith (Hymenoptera: Ichneumonidae). J Insect Behav 11:743–760

Loughrin JH, Manukian A, Heath RR, Tumlinson JH (1995) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. J Chem Ecol 21:1217–1227

McAuslane HJ, Vinson JB, Williams HJ (1991) Influence of adult experience on host microhabitat location by the generalist parasitoid, *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). J Insect Behav 4:101–113

Menzel R, Greggers U, Hammer M (1993) Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In: Papaj DR, Lewis A (eds) Insect learning: ecological and evolutionary perspectives. Chapman & Hall, New York, pp 79–125

Papaj DR, Snellen H, Swaans K, Vet LEM (1994) Unrewarding experiences and their effect on foraging in the parasitic wasp *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). J Insect Behav 7:465–481

Powell W, Pennachio F, Poppy GM, Tremblay E (1998) Strategies involved in the host location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae). Biol Control 11:104–112

Ricard I, Davison AC (2007) Statial inference for olfactometer data. Appl Stat 56:479–492

Steidle JLM, van Loon JJA (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. Entomol Exp Appl 108:133–148

Stireman JO III (2002) Learning in the generalist Tachinid Parasitoid *Exorista mella* Walker (Diptera: Tachinidae). J Insect Behav 15:689–706

Stowe MK, Turlings TCJ, Loughrin JH, Lewis WJ, Tumlinson JH (1995) The chemistry of eavesdropping, alarm, and deceit. Proc Natl Acad Sci 92:23–28

Takabayashi J, Takahashi S, Dicke M, Posthumus MA (1995) Developmental stage of herbivore *Pseudaletia separata* affects production of herbivore-induced synomone by corn plants. J Chem Ecol 3:273–287

Takasu K, Lewis WJ (2003) Learning of host searching cues by the larval parasitoid *Microplitis croceipes*. Entomol Exp Appl 108:77–86

Tamò C, Ricard I, Held M, Davison AC, Turlings TCJ (2006) A comparison of naive and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours. Anim Biol 56:205–220

Tumlinson JH, Turlings TCJ, Lewis WJ (1992) The semiochemical complexes that mediates insect parasitoid foraging. Agric Zool Rev 5:221–252

Turlings TCJ, Benrey B (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. Ecoscience 5:321–333

Turlings TCJ, Wäckers FL (2004) Recruitment of predators and parasitoids by herbivore-damaged plants. In: Cardé RT, Millar J (eds) Advances in insect chemical ecology. Cambridge University Press, Cambridge, pp 21–75

Turlings TCJ, Tumlinson JH, Lewis WJ, Vet LEM (1989) Beneficial arthropod behavior mediated by airborne semiochemicals. VII. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. J Insect Behav 2:217–225

Turlings TCJ, Tumlinson JH, Lewis WJ (1990a) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250:1251–1253

Turlings TCJ, Scheepmaker JWA, Vet LEM, Tumlinson JH, Lewis WJ (1990b) How contact foraging experiences affect the preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). J Chem Ecol 16:1577–1589

Turlings TCJ, Tumlinson JH, Eller FJ, Lewis WJ (1991) Larval-damaged plants—Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. Entomol Exp Appl 58:75–82

Turlings TCJ, Wäckers FL, Vet LEM, Lewis WJ, Tumlinson JH (1993) Learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis A (eds) Insect learning: ecological and evolutionary perspectives. Chapman & Hall, New York, pp 51–78

Turlings TCJ, Loughrin JH, McCall PJ, Röse USR, Lewis WJ, Tumlinson JH (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. Proc Natl Acad Sci 92:4169–4174

Turlings TCJ, Davison AC, Tamò C (2004) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. Physiol Entomol 29:45–55

Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–172

Vet LEM, Lewis WJ, Cardé RT (1995) Parasitoid foraging and learning. In: Cardé RT, Bell WJ (eds) Chemical ecology of insects 2. Chapman & Hall, New York, pp 65–101

Vet LEM, De Jong AG, Franchi E, Papaj DR (1998) The effect of complete versus incomplete information on odor discrimination in a parasitic wasp. Anim Behav 55:1271–1279

Wäckers FL, Lewis WJ (1994) Olfactory and visual learning and their combined influence on the host site location by the parasitoid *Microplitis croceipes*. Biol Control 4:105–112

Wardle AR, Borden JH (1989) Learning of an olfactory stimulus associated with a host microhabitat by *Exeristes roborator*. Entomol Exp Appl 52:271–279